Electrospun Nanofiber Substrates Enhance Oligodendroglial-Directed Differentiation of Neural Stem Cells

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Statement of Purpose: Neural stem/progenitor cells (NSCs) are intrinsically capable of differentiating into different neural cell types within the nervous system, offering prospects for NSC-based cell therapies[1]. Major obstacles still impede the ability to apply neural stem cell therapies clinically, chief among which is the lack of efficient methodologies for controlled differentiation to functional cell types for transplantation. *In vivo*, NSCs are regulated by an array of physical, biochemical and topographical cues that constitute an essential part of their microenvironment. Successful biochemical manipulation of rat adult NSCs has been achieved through supplementation of soluble growth factors to culture medium, for example, to preferentially differentiate a fraction of NSCs to oligodendrocytes with insulin-like growth factor 1 (IGF-1) and noggin[2]. Recently, we have shown 283-nm electrospun fiber substrates significantly promote oligodendrocyte differentiation in comparison to 2D surfaces[3]. Here we investigate the synergistic effects of combining chemical (IGF-1) and topographical (283-nm fibers) cues to obtain a much higher NSC-derived oligodendrocyte population, currently unavailable using either method alone.

Methods: Polyethersulfone (PES) solution was doped with octadecyltrimethylammonium bromide (OTAB) and electrospun to obtain fibers with an average diameter of 283 nm. NSCs were isolated and purified from Fischer 344 rat hippocampi. Cells were seeded (2000 cells/well) onto scaffolds in 24 well plates and TCPS (2-D control) which had been coated with PLO/Laminin for NSC adhesion[3]. Cells were cultured in medium containing 1 ng/mL fibroblast growth factor-2 (FGF-2) to maintain cell viability. After 24 h, IGF-1 and noggin were added to solution to obtain a concentration of 500 ng/mL each. Cells were cultured for 4 d, then fixed and stained for immunofluorescent analysis. Nestin (NSC marker), Tuj-1 (neurons), GFAP (astrocytes), RIP (oligodendrocytes) and DAPI (nucleus) staining was performed. A separate set was fixed and ethanol/hexamethyldisilizane-dehydrated for SEM imaging.

Results: When NSCs were cultured on 283-nm nanofibers in the presence of IGF-1 and Noggin, over 90% of cells showed RIP+ staining, compared to less than 25% among cells cultured on the TCPS substrates. In addition, cells cultured on nanofiber substrates expressed RIP at a much higher intensity than their TCPS counterparts. Correspondingly, a small (< 15%) fraction of cells cultured on nanofibers were nestin+, compared to nearly 100% nestin+ staining for NSCs cultured on TCPS, clearly indicating an enhanced differentiation towards the oligodendrocyte lineage as a result of a synergistic effect between the biochemical and topographical cues. Tuj-1 and GFAP staining was largely negative (< 5%) in each case, implying a strong stimulus preferentially driven towards oligodendrocyte differentiation. SEM results revealed drastically different cellular morphologies between cells cultured on the two substrates. NSCs cultured on nanofibers had a very flat and stretched profile, extending in all directions and following the underlying nanofibers, indicating strong contact guidance as well as a likely restricted cellular mobility. NSCs cultured on the TCPS surface were smaller and did not show extensive dendrite formation typical of glial cells. There were multiple attachment points, but not nearly as much substrate interaction as seen in the fiber samples.

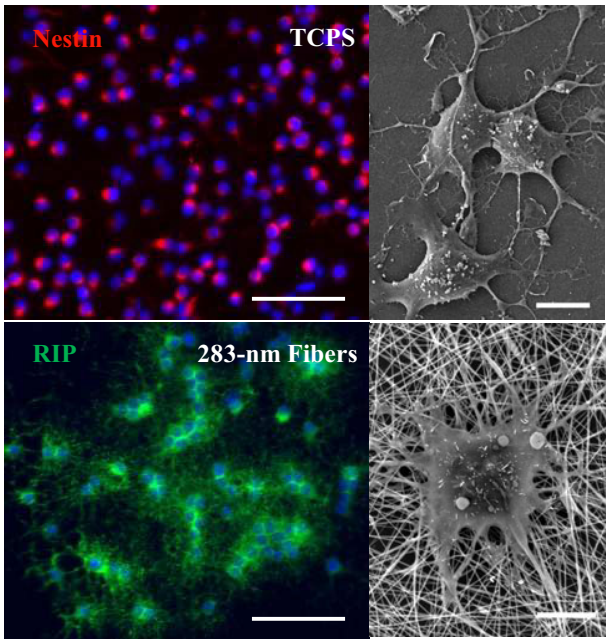

Figure 1. Representative immunofluorescent and SEM images of neural stem cells cultured with 500 ng/mL noggin and 500 ng/mL IGF-1. Cell nuclei are counterstained with DAPI (blue). Scale bars are 100 μm (IF) and 10 μm (SEM).

Conclusions: We have shown that combining IGF-1 with 283-nm fiber substrates is extremely effective in directing NSCs to differentiate into oligodendrocytes. The combination of biochemical and topographical cues is more efficient in controlling cellular fate than either method alone, and raises important questions regarding fate-specification mechanisms enhanced by substrate topography. Further studies are ongoing to unravel signaling mechanisms involved.

References:

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